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April 30, 2014

Marcia Bailey
US EPA
Region 10

ASSISTANCE REQUESTED:	External Peer Review Comments on the Study Reports by Huntingdon Life Sciences (2001a and 2001b) related to Sulfolane PPRTV
ENCLOSED INFORMATION:	**Attachment 1**: External Peer Review Comments on the Study Reports by Huntingdon Life Sciences (2001a and 2001b): Sulfolane Toxicity Study by Oral Administration via Drinking Water to Cd Rats for 13 Weeks, Volumes I and II

If you have any questions regarding this transmission, please contact the STSC at (513) 569-7300.

Attachments (1)

cc: STSC files

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External Peer Review Comments on the Study Reports by Huntingdon Life Sciences (2001a and 2001b): Sulfolane Toxicity Study by Oral Administration via Drinking Water to Cd Rats for 13 Weeks, Volumes I and II

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Contents

PEER REVIEW COMMENTS FROM

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Note: Ms. Iannucci conducted this review as an independent consultant and not as a representative of OSHA.

External Peer Review Comments on the Study Reports by Huntingdon Life Sciences (2001a and 2001b): Sulfolane Toxicity Study by Oral Administration via Drinking Water to Cd Rats for 13 Weeks, Volumes I and II

Responses to Charge Questions from Ms. Annette Iannucci

1. **Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, dietary exposure, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?**

This experimental study was properly designed according to OECD Guideline 408 (1998) – Repeat Dose 90-Day Oral Toxicity Study in Rodents (http://browse.oecdbookshop.org/oecd/pdfs/free/9740801e.pdf). OECD Guideline 408 is very consistent with EPA Guideline OPPTS 870.3100 (1998) – 90-Day Oral Toxicity Study in Rodents. This study met OECD Guideline 408 requirements for dosing duration, oral dosing through drinking water, animal species used, number of animals per dose level and sex, and endpoints examined (i.e., clinical observations, body weight gain, food and water intake, functional observational battery (FOB), hematology, clinical chemistry, gross necropsy, organ weight, and histopathology). The dose range was adequate because it included at least one dose that resulted in a no observed effect level (NOEL), and higher doses did not cause severe toxicity (e.g., death). Results were analyzed for statistical significance. The study was conducted according to good laboratory practices (GLP), and the report included a quality assurance statement that explained the types of audits conducted. Accuracy and stability of dosing solutions were verified.

One limitation noted was that the study did not report the purity of the test compound.

2. **Are there physiological/toxicological endpoints that should have been assessed that were not part of the investigation?**

The study examined endpoints required by OECD Guideline 408. However, as will be discussed in greater detail below, determining and reporting the estrous stage of females at necropsy would have been useful for interpreting uterine effects.

3. **Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?**

Overall, the animal data were correctly summarized. In the results section, the study authors reported both statistically significant findings, as well as findings for which there appeared to be a trend, but statistical significance was not achieved. A few limitations were noted regarding reporting of possible effects.

Clinical signs data were only reported by individual animal and not by numbers of animals affected per group. The lack of group incidence rates made it impractical to verify the authors' conclusions that there were no clinical signs related to treatment. However, clinical signs data were summarized per group in the FOB report, and it was possible to verify lack of clinical signs in that report.

Table 10 indicated a slightly higher incidence of renal corticomedullary mineralization in females of the

1600 mg/L group compared to females in the control or other treatment groups (n= 3/10 versus 0–1/10). This effect should have been discussed in the Results section.

Some other histopathology findings listed in Table 10 appeared to occur in slightly more animals of the 1600 mg/L compared to the control group but were not discussed in the Results section. Those findings included aggregation of alveolar macrophage in lungs/bronchi of females (n= 3/10 in 1600 mg/L group versus 0–1/10 in control and other dose groups) and stromal fatty infiltration in pancreas of males (2/10 in 1600 mg/L group versus 0/10 in other dose groups).

Overall, the data were correctly interpreted, but some questions regarding study authors' data interpretation were noted.

The study authors concluded that a number of statistically significant hematology findings represented normal biological variation because the effects were minor or lacked dose-response relationships. Those effects included prolonged prothrombin times in males, increased mean cell volumes and reduced activated partial thromboplastin time in females, and reduced basophil counts in males and females. Because hematology effects such as low lymphocyte, monocyte, and large unstained cell counts were considered to be treatment-related effects in females, it would seem reasonable that other blood cells could have been a target for sulfolane toxicity. Therefore, the study authors should have provided more information, such has historical control data, to support their conclusion.

The study authors concluded that decreased aspartate amino transferase activity in high dose males was not treatment related because the control mean was increased as a result of unusually high values in two of the control animals. The study authors should have provided more information to support their conclusion, such as historical control values.

The study authors claimed that the absolute and relative uterine weight increases in the 100 and 1600 mg/L groups resulted from increased incidence of uterine fluid distention related to estrous stage. Although absolute and relative uterus weights were higher in the 1600 mg/L group than in 100 mg/L group, the number of females exhibiting uterine fluid distention was greater in the 100 mg/L than in the 1600 mg/L group (4 of 10 versus 2 of 10). Based on the incidences of uterine fluid distention, it would be expected that uterine weight would have been higher in the 100 mg/L group. The Materials and Methods section did not mention if or how the estrous stage of females was determined, and no data table reported the numbers of females at each estrous stage at necropsy. According to Table 10, which reports histopathology findings, the incidence of uterine luminal dilatation was also increased in the 100 and 1600 mg/L groups (4/10 and 5/10, respectively). The study authors should have discussed whether the uterine luminal dilatation findings were possibly related to estrous cycle or sulfolane treatment.

4. Are the conclusions and the discussion in the report supported by the data? Were there critical results or issues that were not discussed or addressed in the results or conclusion? Were there any contradictory statements or observations in the study regarding Sulfolane?

Examples of findings not reported in the Results section, or of questionable interpretations by study authors are given in Question #3 above. Most of the study authors' interpretations would not affect selection of target organs, but data were insufficient to rule out a possible effect of sulfolane on the uterus or endocrine system. None of the questionable interpretations would affect selection of NOELs or LOELs and they are, therefore, not considered critical limitations.

The Discussion section suggested that a finding of reduced alanine amino transferase is usually not considered to be toxicologically significant. However, the study authors did not provide a reference to support that statement.

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

This Huntingdon Life Sciences study on sulfolane toxicity is a quality study which was properly planned, conducted, and reported. The quality of the study would have been improved if estrous stage at necropsy was determined and reported. Although not required by OECD Guideline 408, determining the estrous stage of females would have been useful for interpreting the uterine effects observed. Some limitations were noted regarding reporting of or conclusions about findings, but the report presented data in sufficient detail to allow readers to reach their own conclusions.

PEER REVIEW COMMENTS FROM

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External Peer Review Comments on the Study Reports by Huntingdon Life Sciences (2001a and 2001b): Sulfolane Toxicity Study by Oral Administration via Drinking Water to Cd Rats for 13 Weeks, Volumes I and II

Responses to Charge Questions from Dr. Sam Kacew

1. **Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, dietary exposure, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?**

With respect to a 13-week subchronic oral drinking water toxicity evaluation of sulfolane on rats the protocols employed are in accordance with Good Lab Practice (GLP); the animals were treated humanely in accordance with the Geneva conventions, and met the requirements stipulated by the Organisation for Economic Co-operation and Development (OECD) for testing of chemicals. As stipulated there were 10 animals per group of both genders (male and female). The choice of species and strain CD rat was appropriate and justifiable to examine the potential oral drinking water toxicity of sulfolane based upon regulatory agency requirements and because of historical control database to enable interpolation form rat to human for risk assessment purposes. The oral exposure to sulfolane via drinking water is appropriate as an oral route but this is quite distinct from an oral dietary exposure. As the title clearly states this is "Sulfolane toxicity study by oral administration via the drinking water to CD rats for 13 weeks". In the Background to the Charge it states the "report is on the effects from subchronic oral (dietary) exposure to Sulfolane in CD rats". This statement is not correct as exposure to a chemical in drinking water is different and distinct from exposure to a chemical in the food. Although both are oral the vehicle water is different from the vehicle food and one might observe differences in the results. In this study water consumption rather than food consumption is the critical factor.

In an oral drinking water study the quantity of water imbibed is an essential component with respect to the amount if chemical that enters the organism. In this system the amount of food consumed does not affect the level of chemical exposure directly. In an oral drinking water study an important factor is the taste. If a chemical in the drinking water produces an aversion due to taste then subsequently the animal will drink less, which would lower the quantity of chemical imbibed. The rat may eat less and this would result in a lower bodyweight. Thus a bodyweight change is not a direct consequence of the chemical but a secondary effect as a result of taste aversion subsequently followed by less food ingested.

In a dietary study the quantity of chemical entering the organism is dependent on the amount of food consumed and not related to the quantity of drinking water imbibed. Hence this was not a dietary exposure to sulfolane. In order to conduct a dietary exposure the chemical must be mixed into the feed in known amounts, the amount of feed consumed determined and subsequently the dosage calculated.

In both a drinking water and dietary subchronic oral exposure study, it is essential to measure chemical concentrations in blood, urine and tissues to correlate chemical levels with potential adverse effects/endpoints.

In general, the endpoints examined for a 13 week subchronic toxicity study at a gross or macroscopic screening level were appropriate and justifiable. However, one of the aims of the study was to correlate chemical exposure via water consumption to toxicity endpoints. In this study drinking water containing varying amounts of chemical was supplied to a cage containing 5rats/gender/group. Water consumption was measured/cage and this was divided by 5 assuming each rat drank an equal amount of water. This is a crude measure and not precise to obtain an "achieved overall dose". In addition, each rat has a different excretory rate such that the amount of chemical in the organism on a daily basis would be the amount of

chemical consumed minus the amount of chemical excreted. The water consumption measure thus does not reflect an achieved dosage and is not accurate. The study design should have included metabolic cages where one clearly determines the amount of chemical consumed via drinking water and the quantity of chemical excreted in the urine. In addition, the chemical could have been determined in the blood samples at termination for correlation between amount of chemical within the organism and the endpoint studied. Further, chemical tissue levels especially the kidney could have been determined. The water and food consumption and certainly the achieved dosages are not accurate measures and limit any usefulness to correlate chemical concentration to response. Data from week 1 inferred a fall in bodyweight gain; however this cannot be attributed solely to chemical as taste aversion rather than chemical may have been the endpoint measured.

With respect to the measurement of biochemical parameters the use of plasma is unusual. It should be emphasized that plasma and serum are uniquely different. In addition, the historical database for clinical chemistries such as ALT, GPT, glucose, urea, total cholesterol, creatinine, urea, sodium and potassium are based upon measures determined in serum and NOT plasma. It is questionable whether the plasma was indeed used. If plasma was employed as the body constituent then the historical database cannot be used for comparative purposes as the data were generated from serum.

A historical database for endpoint values for these parameters as a control range for plasma levels needs to be provided. A clear problem lies in the Control data for AST in both genders. If one examines Appendix 10 and the control data for males the AST values range from 71 to 246, which is a 3-fold difference within control. In control females the AST value ranges from 59 to 154 a 2-fold variation. With such high variability one can not adequately assess whether a chemical affects this plasma enzyme. In control females plasma ALT ranged from 26 to 153 a 5-fold difference again negating the possibility of determining a chemical-induced effect. The control data for AST and ALT in plasma are thus questionable and serum should have been used as the organism constituent.

With respect to the hematological parameters, it is clear that basophil measurements are not appropriate. The values provided are either not detectable (00.0) or barely detectable/non-detectable (0.01). The latter values appear as significant but are NOT appropriate as it appears the instrumentation was not sufficiently sensitive to determine basophil numbers and should not be included. The authors need to provide the control historical range for all hematological data especially if one examines Appendix 9 for male WBC, neutrophil and lymphocyte data for animal 14. In essence these data seem to be an outlier and as data from male # 15 is CTD, thus the N for this group was 9. The inclusion of the control outlier may have affected observation of a chemical-induced effect. It is important to include the range of historical data for hematological values to ensure that the outlier may indeed fall into the normal range or additional control data are required. If one looks at the control male neutrophil data the range is from 0.41 to 2.11 (5-fold) and for lymphocytes the value of 4.5 to 11.6 (3-fold) and in control females WBC from 4.8 to 12.1 (2-fold) and lymphocytes from 4.2 to 11.1 (3-fold) can affect determination of chemical-induced effects. This emphasizes the need for insertion of range of control data hematological parameters.

The terminal procedure of sacrifice by carbon dioxide inhalation is appropriate and humane. The removal of tissues for necropsy and subsequent histopathology preparations were conducted in accordance with GLP procedures. Microscopic examination of tissues and grading of responses was conducted by trained, expert pathologists and observations are appropriate.

The statistical analyses with respect to the FOB numerical data are well-described, easy to follow and appropriate. Statistical analyses with respect to organ weights, bodyweights as well as macroscopic and microscopic pathology at $p<0.05$ are easy to follow, well-described and justifiable. However, the description of statistical analysis for hematological parameters and clinical chemistries on page 21 does not correspond to the statistical tests used in Tables 6 & 7. The test on page 21 describes a Bartlett's test for hematology and clinical chemistries at the $p<0.05$ level. There was no mention of a William's test or a Shirley's test. In addition, data were subjected to both $p<0.05$ and $p< 0.01$. There is no justification

provided for the basis for the use of $p<0.01$. Does this infer differences in statistical and more importantly biological relevance? Further if one examine the Na data in Table 7 for males, it states that Na for control at 141 SD 1.1 is significant from 400 mg/L value of 140 SD 0.9 and 1600 mg/L value 138 SD 5.1. This is statistically NOT possible at $p<0.05$ or $p<0.01$. This also applies to females in Table 7 where it is NOT statistically possible for any values for Na to be significant from control yet this is indicated at 25, 100, 400 and 1600 mg/L. If one looks at Table 7 at male AST for control at 10 SD 55 vs 1600 mg/L value of 68 SD 10 it states significance at $p<0.01$. This again is NOT statistically possible. In addition the value for control male creatinine of 49 SD 3.5 vs the value at 1600 mg/L of 53 SD 1.8 is not statistically possible at the $p<0.01$ level. Similarly for males the basophil control data of 0.02 SD 007 vs 0.01 at 400 and 1600 mg/L can NOT be statistically significant at $p<0.01$ in Table 6. The basophil data for females in Table 6 at 100, 400 and 1600 mg/L is NOT possible. An instrument does not provide 0.00 as a measure as this simply not detectable and thus not comparable statistically. The use of statistical analysis for blood chemistries and hematology is questionable for some parameters.

The experiments were conducted appropriately and quality assurance was maintained throughout the study.

2. Are there physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

As this constituted a subchronic 13-week oral drinking water study on the effects of sulfolane on toxicity in CD rats the individual imbibition and excretion of chemical in each animal should have been determined in order to obtain chemical concentrations in the animal. This is especially critical for determination of "achieved dosage". In serum the measurement of triglycerides, gamma-glutamyl transpeptidase, lactate dehydrogenase and alkaline phosphatase should have been determined. With the use of metabolic cages additional parameters including the measurement in urine of levels of chemical, glucose, protein, bilirubin and creatinine; pH and specific gravity as well as urinary enzymes such as alkaline phosphatase should have been measured. At termination the blood sample obtained could have been employed for the determination of chemical levels.

3. Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

The strength of the study lies in the findings that a 13-week oral drinking water investigation did not produce any apparent gross macroscopic (hematology and blood chemistries) or microscopic (histopathological alterations) in both genders in a concentration-dependent manner. However, one can not ascribe a specific chemical concentration to a specific endpoint response as chemical concentrations were not determined in individual animals and exposure to chemical was on a per 5 rat/cage basis. It is presumed that even within a cage of 5 rats that each rat imbibed some water containing the chemical but the quantity is not known on an individual basis. Further, no animals died within each cage of 5 rats at all chemical dose levels and there were no significant changes in bodyweights, tissue weights, FOB, ocular changes and histopathology. In addition, the clinical chemistries reported to be altered slightly are questionable as plasma rather than serum was used and the range of control values was quite variable in some cases. The control hematology values especially for lymphocytes, basophils and neutrophils raised the relevance of any perceived significant effects reported. The use of a 5rat/cage model to establish a correlation between chemical and toxicological endpoint used here was not appropriate and the large variability in control data for some parameters diminished both strength and credibility. The individual

data summarized on page 8 for bodyweight as "low" has no biological meaning or relevance especially as there were no other bodyweight changes throughout the study. The conclusion that lymphocyte number was low again is questionable as there was high variability in the control group. The conclusion of slightly changed for ALT is misleading due to the high variability in control data for ALT especially in females and the questionable AST data. The relevance of slightly changed for creatinine in males is questionable statistically and further diminished in light of lack of alterations in Na and K and no changes in renal pathology. The data from the individual animals was over-interpreted to suggest that sulfolane produced any relevant biological effects, in view of the wide range of values within controls for ALT and AST and the use of plasma rather than serum for enzymes. In fact some of the statistical applications to basophils, Na and creatinine levels were not accurate.

4. Are the conclusions and the discussion in the report supported by the data? Were there critical results or issues that were not discussed or addressed in the results or conclusion? Were there any contradictory statements or observations in the study regarding Sulfolane?

There are portions of the conclusions and discussion that are not reflective of the results. On page 23 in the description of Organ weights with respect to the uterus the authors conclude that the changes observed are due to a slight increased incidence of uterine fluid retention due to the stage of oestrus. There is no evidence presented to show any differences in fluid quantity and the stage of the reproductive cycle were not measured. This speculation is not based upon scientific data in this study.

On page 25 in the Discussion it states that leucocytes were a target of toxicity. Based upon the wide variability and range of control WBC and lymphocyte data it is not evident that results from the 1600mg/L are outside the control range. In addition, there are no histopathological alterations in spleen, bone marrow and thymus and hematopoietic parameters to suggest any toxicity in the hematology or immune system. A closer scrutiny of the data might reveal that the data in treated animals actually falls within the normal range. The conclusion that leucocytes are a target for toxicity is not supported by the results presented.

The conclusion that low bodyweight gain and food conversion efficiency during the first week are considered to represent non-specific indicators of toxicity are not supported by the results. There were no apparent signs of toxicity following gross examination of animals. All rats survived and bodyweight gain was not affected by any treatment for weeks 2-13. In addition, the study failed to examine the taste of the drinking water which may have resulted in aversion to drinking the water and thus less food consumed. Clearly this parameter was not indicative of any toxicity.

The conclusion that the kidney is a target and that sulfolane produced hyaline droplets is in agreement with solvent induced "hydrocarbon nephropathy". This finding occurred in males and is not relevant for humans. The increased plasma creatinine concentrations are based upon inaccurate statistical analysis. In addition, the lack of effects on plasma Na and K and no histopathological alterations indicate that the kidney was still able to carry out its functions. There was no evidence of basophilia at 1600mg/L as the data were not detectable and the instrumentation lacked sensitivity and was below the limit of detection. This conclusion based upon the results was not justifiable.

The conclusion that 1600 mg/L reduced plasma alanine amino-transferase (ALT) is questionable as this enzyme is normally measured in serum and not plasma. Further if one closely examines the range of control values then the treated values fall within the historical range indicating lack of a relevant effect. The conclusion that changes in ALT lack toxicological and biological significance is clearly justified but it is questionable that data show there was a "slight reduction".

With respect to the equivalent dosage, the experimental protocol was designed in a manner that this could not be determined. The chemical was supplied to the drinking water of 5 animals /cage. One can not assume

that all 5 rats will imbibe an equal amount of water even though the weight gain is equivalent. In addition, the chemical concentrations were not determined in the animal. In essence what is presented in this study is a "presumed equivalent dose" and NOT an achieved dose.

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

In order to correlate the effects of chemicals at a specific concentration to specific endpoints then the experimental protocol using drinking water should be designed in a manner where the amount of chemical in the vehicle is known and the quantity of water imbibed by the animal is known and the amount of water excreted as urine is measured. The manner in which this protocol is achieved is with the use of metabolic cages, which was not employed in this investigation. However, if this is not the case as in this study with 5 rats /cage, then the concentration of chemical needs to be determined within the animal. This is accomplished by obtaining a blood sample and measuring the chemical. This was not done in this study. The study was poorly planned in that there was no urine collected for urinalysis especially in the view that sulfolane is a hydrocarbon belonging to a category of chemicals known to induce "hydrocarbon nephropathy".

With respect to clinical chemistries, the assays are conducted on serum samples. It is not clear why plasma was selected especially since the historical database is comprised of serum values. The measurement of hematological parameters such as basophils was below the instrument limits of detection and should not have been reported and certainly not subjected to statistical analysis. The statistical analysis used in this study was not reflective of the tests in the tables where a William's test and a Shirley's test appear but are not stated in the text. In addition, data in the text for Na, creatinine and AST were shown as significant but this was not possible when one examines the data. The usage of terminology such as "slight" or "low" which have no biological and toxicological meaning detracted from the interpretation of what the data meant in reality.

The quality of this investigation would have been enhanced with the inclusion of blood and kidney chemical tissue levels. A urinalysis would have provided more important information on renal functions. The use of metabolic cages would have achieved the ability to correlated endpoints to the amount of exposure to chemical. The addition of historical endpoint values for control would have enabled one to verify whether the treated values observed were in reality within control range levels.

PEER REVIEW COMMENTS FROM

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External Peer Review Comments on the Study Reports by Huntingdon Life Sciences (2001a and 2001b): Sulfolane Toxicity Study by Oral Administration via Drinking Water to Cd Rats for 13 Weeks, Volumes I and II

Responses to Charge Questions from Dr. Lawrence H. Lash

1. Based on your knowledge of toxicological protocols, please comment on the experimental design of this investigation. Do you see any significant issues with the test system or test article employed, dietary exposure, endpoints recorded, terminal procedures, statistical analyses, and quality assurance?

The experimental design is presented in great detail and addresses all key issues relating to animal care, the test article, dietary exposure (including calculation of precise exposure doses with appropriate correction factors), details of all the endpoints that were recorded (including how the endpoints were recorded, standardized, and instrument calibration), terminal procedures, statistical analyses for the different types of data obtained (i.e., continuous and categorical), and quality assurance. Suites of biochemical assays in blood and select tissues, histopathology and measurements of basic tissue weights were studied over the 13-week period of the exposures. I see no issues or concerns with any of these aspects of the study.

2. Are there physiological/toxicological endpoints that should have been assessed that were not part of the investigation?

Based on a literature search on PubMed I performed on September 8, 2011 (see listing of search results under Question #5), a previous study of sulfolane toxicity in several species (Brown et al., 1966) investigated potential skin irritant properties of sulfolane. Although results were negative and doses of sulfolane that were used were markedly (~ 2-3 orders of magnitude) higher than those used in the present study, it may have been advisable to assess this possible, adverse effect, at least on a limited basis. The previous study was of a short-term exposure; different results may conceivably be possible with the 13-week, subchronic exposure. Otherwise, all the basic tests of key organ function and histopathology were complete.

3. Please comment on the strength, credibility, and relevance of the toxicological results. Were the individual animal data correctly summarized and interpreted?

Presentations, summaries, analyses, and interpretations of the data were thorough, presented with proper rationale and explanation. A reasonable number of animals were assigned to each treatment group and the data show a good deal of consistency, i.e., a small amount of variation. The only concern I noted was the use of the term "signs" (Vol. I, pp. 24, 29, 30 and elsewhere) to describe the basic observations of animal health that were routinely conducted. This seems to be an odd term to describe what was measured. A better descriptor (e.g., "Overall physical examination") should be used.

4. Are the conclusions and the discussion in the report supported by the data? Were there critical results or issues that were not discussed or addressed in the results or conclusion? Were there any contradictory statements or observations in the study regarding Sulfolane?

In general, the experimental design, results, and conclusions derived from the results are clearly presented and discussed. All critical issues relating to the data were properly considered. A few statements were made that could, however, be better stated.

- On pp. 17 of Volume I, it is stated that "there were no signs related to treatment." As explained in the response to Question #3 above, this is a vague and confusing term and should be replaced with a better descriptor.
- On pp. 22 of Volume I (and elsewhere), the document describes the method used to allocate animals to treatment groups as being "non-selective." Although this term is technically correct, most people would state that animals were "randomly assigned to groups."
- On pp. 35 of Volume I, the document states: "In females given 100 mg/L or more there was a clear reduction of lymphocyte, monocyte and large unstained cell counts, though this did not follow a strong trend with dosage." The meaning of this conclusion is a bit unclear. Does a lack of dose dependency imply that some artifact existed? Is the finding toxicologically important?

5. In your opinion, was this investigation properly planned, conducted, and reported? Are there any procedures, observations or analyses that would have added to the quality of this investigation?

The documents present detailed explanations of how the study was planned and conducted. Detailed time lines for each phase of the study and for reporting of results are presented. The reports are thorough and well organized, showing both summary data and detailed data for each animal in each treatment group.

Although this is not a risk assessment document per se, addition of some review of the literature on the potential toxicity of sulfolane could have been added to help place the experimental design and results in the its fullest possible context. A search of the PubMed database on September 8, 2011, using the search term "sulfolane," resulted in a total of 87 citations. Of these publications, 11 were deemed to be relevant in terms of covering potential adverse or toxic effects of sulfolane. Of particular note is that exposure doses used in several of these studies, particularly the behavioral and neurotoxicity studies, were 1- to 2-orders of magnitude higher than the highest dose used in this study. Thus, while the effects (e.g., hypothermia) are not likely to be observed at the doses used in this study, mention of these other data would be appropriate for completeness.

Relevant Publications from PubMed Search (in chronological order):

1. Brown, V.K.H., Ferrigan, L.W., and Stevenson, D.E. (1966) Acute toxicity and skin irritant properties of sulfolane. Brit. J. Industr. Med. 23, 302-304.
2. Andersen, M.E., Jones, R.A., Kurlansik, L., Mehl, R.G., and Jenkins, L.J. Jr. (1976) Sulfolane-induced convulsions in rodents. Res. Commun. Chem. Pathol. Pharmacol. 15, 571-580.
3. Andersen, M.E., Jones, R.A., Mehl, R.G., Hill, T.A., Kurlansik, L., and Jenkins, L.J. Jr. (1977) The inhalation toxicity of sulfolane (tetrahydrothiophene-1,1-dioxide). Toxicol. App. Pharmacol. 40, 463-472.

4. Gordon, C.J., Long, M.D., and Dyer, R.S. (1984) Effect of ambient temperature on the hypometabolic and hypothermic effects of sulfolane in rats. Arch. Toxicol. 56, 123-127.

5. Gordon, C.J., Dyer, R.S., Long, M.D., and Fehlner, K.S. (1985) Effect of sulfolane on behavioral and autonomic thermoregulation in the rat. J. Toxicol. Environ. Health 16, 461-468.

6. Ruppert, P.H., and Dyer, R.S. (1985) Acute behavioral toxicity of sulfolane: influence of hypothermia. Toxicol. Lett. 28, 111-116.

7. Gordon, C.J., Long, M.D., Fehlner, K.S., and Dyer, R.S. (1986) Sulfolane-induced hypothermia enhances survivability in mice. Environ. Res. 40, 92-97.

8. Burdette, L.J., and Dyer, R.S. (1986) Sulfolane effects on audiogenic, pentylenetetrazol and afterdischarge seizure activity. Neurobehav. Toxicol. Teratol. 8, 621-626.

9. Dyer, R.S., Boyes, W.K., and Hetzler, B.E. (1986) Acute sulfolane exposure produces temperature-independent and dependent changes in visual evoked potentials. Neurobehav. Toxicol. Teratol. 8, 687-693.

10. Mohler, F.S., and Gordon, C.J. (1988) Thermoregulatory responses of the rabbit to subcutaneous injection of sulfolane. Arch. Toxicol. 62, 216-219.

11. Ogurtsov, N.A., and Vysotskaia, N.A. (1992) [The mechanism of the radioprotective action of sulfolane]. Radiobiologiia 32, 130-133. [Article in Russian]